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MICROPROPAGATION OF VAJRADANTI (*BARLERIA PRIONITIS* L.): A HIGH VALUE ANTIDONTALGIC AND ETHNO-MEDICINAL HERB

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Tissue culture of medicinal plants is widely used to produce clones and has been found in the conservation of threatened plants species face risk of extinction. Vajradanti is an ethno-medicinally important plant showing pharmacological effects such as anti-microbial, anti-helminthic, anti-fertility, anti-oxidant, anti-diabetic, anti-arthritic, hepato-protective, diuretic, cytoprotective, antidiarrhoeal, analgesic, anti-leukemic, anti-inflammatory and hypoglycemic properties. Micropropagation in most of the plant species involve proliferation of explants using MS medium. There are scanty reports on tissue culture and regeneration of Vajradanti. However, no effective *in vitro* regeneration protocol has been developed for this medicinally important plant. Development of high frequency regeneration pathway was undertaken using shoot tip and nodal segments of *Barleria prionitis* on MS medium supplemented with auxin and cytokinins alone and in various combinations.

Keywords: Conservation; Micropropagation; Regeneration; Vajradanti.

Introduction

Barleria prionitis (L.) is an ethno-medicinally important herb commonly called Porcupine flower, belonging to family Acanthaceae. *Barleria* is a large, widespread, polymorphic, pan tropical genus comprised of approx. 300 species of herbs and shrubs. Because of its anti-dontalgic properties it is also known as 'Vajradanti'[1]. Whole plant of *B. prionitis* is used in many ayurvedic formulations and preparations like *Rasnadi Kvatha, Rasnadi Churna, Sahachara Ghritha, Sahachara Taila* and *Dantaroganashani* Churna[1,2]. People of many tribals of India-Bheel and Garasia, chew the leaves to relive body ache, toothache and to cure bleeding gums; they use dried powder of flowers with sugar to make a paste called *Gulkand* which is taken orally for few days to cure painful menstruation. *B. prionitis* is widely distributed throughout Africa, India, Sri Lanka and tropical Asia[3]. It is planted as an ornamental and cultivated plant in Asia[4]. *Barleria* is considered as an endangered species[5-7]. *Barleria* possesses undoubtedly a number of medicinal properties and widely used in treatment of gastro-intestinal disorders, constipation, intestinal worms, digestive troubles, liver disorders, whooping cough, toothache[4], respiratory syncytial and joint pain[8]. The International Union for Conservation of Nature (IUCN) Species survival commission report ''Extinction

crisis continues apace'' for the year 2009 revealed that 70% of plants are under threat. This new era of medicine world immediate rising demand of plant-based drugs is unfortunately creating a pressure on some important medicinal plant populations. High demand and over-exploitation of this species by local ayurvedic practitioners, this plant species is likely to be under threat very soon.

Plant tissue culture technique can provide an alternate source of ex situ conservation and large-scale production of many medicinally important and endangered species[9,10]. In recent years, uses of plant growth regulators in in vitro techniques have been found increasing for conservation of high value plants. *Barleria* exhibits very low percentage of seed viability[11]. In recent years, many workers have made efforts to develop *in vitro* micropropagation protocols[7]. MS[12] medium is generally used for the plant propagation through shoot tip and leaf explants on various concentrations of plant growth regulators. A protocol for callus induction has been reported by[12-14]. In a review, a detailed study has been provided regarding the fast depletion of plant resources and their present status[18].

The present investigation deals with shoot induction and vegetative propagation of *Vajradanti* under *in vitro* conditions, and exploring the effect of different plant growth regulators.

Shot tips were inoculated on MS medium supplemented with BAP, Kn and coconut water in various concentrations and combinations. Maximum (96%) shoot induction or bud break was obtained on MS medium supplemented with 8.88 µM BAP giving an average of 3.82±0.13 shoots per explant and shoot length approx 1.74±0.08 cm. The elongated shoots were excised from mother explant and further multiplied on MS medium supplemented with 4.44 µM BAP.

Material and methods

Collection of plant material and surface sterilization: Wild plants of *Barleria*, growing in the forest of Shahabad town near Baran district of Rajasthan were identified as source plants for experimentation. Plants were also grown in the earthen pots so as to monitor the growth and to collect plant material for further experiments.

Healthy shoot cuttings were excised from 4-5 year old plant. After excision, the plant material was washed under running tap water for 10-15 min and then washed with 0.1% Labolene (Qualigens-Fisher) mild detergent. Plant material was also subjected to treatment with antifungal and antibacterial agent (Bacitracin; Hi Media). The plant material was then surface sterilized with 70 % ethanol for 30 sec. and again rinsed twice with double distilled water. Further sterilization of plant material was carried out by 0.1% $HgCl_2$, for 3 min. and washed 5 times with autoclaved distilled water under laminar air flow hood. The medium containing 3% sucrose was solidified with 0.8-1 % agar (Hi Media). pH of the nutrient medium was adjusted to 5.8±0.2. Medium was sterilized by autoclaving at 121°C and 15 psi pressure for 15-20 minutes after dispensing in 100 ml Erlenmeyer flasks and plugged with non absorbent sterile cotton. Shoot tips containing shoot apex (1.0-1.5 mm) were transferred in culture tubes containing MS medium supplemented with 6-benzyl aminopurine (BAP), Kinetin (Kn) and α-naphthaleneacetic acid (NAA) alone or in combinations.

All culture vessels were incubated at temperature 26±2°C with a relative humidity of 50-60% and a photoperiod of 16h per day at 2000-2500 lux provided by fluorescent

incandescent tubes. For each experiments a minimum 7 replicates were taken and repeated thrice.

The nodal segments were also cultured on nutrient medium supplemented with BAP, Kn and NAA in the same manner as described above for shoot tips.

Results and Discussion

In the recent years, very expressive tissue culture and micro-propagation studies have been carried out in various plant species of family Acanthaceae.

Table 1. Response of bud break from cultured shoot tip of Vajradanti (*Barleria prionitis* L.) on MS medium supplemented with BAP

Hormone Concentration BAP/ Kn (mg/ L)	No. of explants cultured	No. of explants responded	Response type
BAP			
0.0 (Control)	25	9	+
0.5	25	18	+++
1.0	25	19	+++
1.5	25	21	++++
2.5	25	18	+++
4.0	25	17	+++
Kn			
0.5	25	18	+++
1.0	25	22	++++
1.5	25	20	+++
2.5	25	19	+++|
4.0	25	17	+++

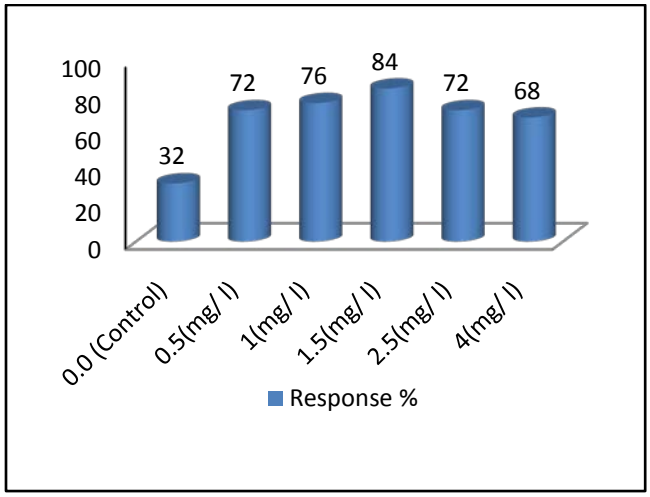

Fig.1. A *Barleria prionitis*; graph showing percentage response of shoot tip cultured on MS medium supplemented with BAP

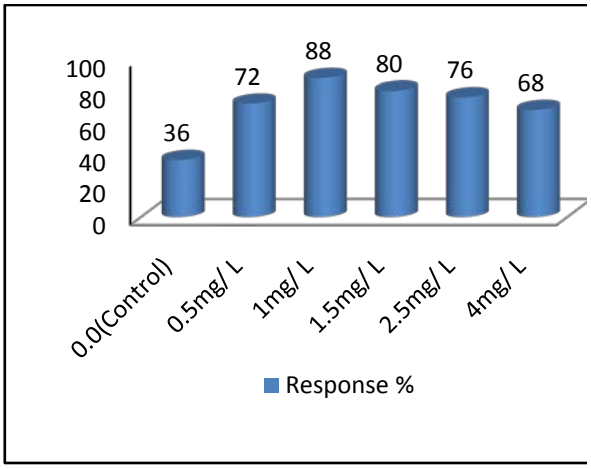

Fig.1. B *Barleria prionitis*; graph showing percentage response of shoot tip cultured on MS medium supplemented with Kn

Table 2. Interactive effect of BAP/Kn and NAA on shoot induction from shoot tip of *Barleria*.

Hormone Conc. (mg/ l)		No. of explants cultured	No. of explants responded	Response type	Response (%)
BAP	**NAA**				
0.0	0.0	25	9	+	36
1.5	0.0	25	3	+	12
1.5	0.5	25	18	+++	72
1.5	**1.0**	**25**	**24**	**++++**	**96**
1.5	1.5	25	20	+++	80
1.5	2.0	25	19	+++	76
1.5	2.5	25	16	+++	64
Kn	**NAA**				
1.0	0.0	25	12	+++	48
1.0	**0.5**	**25**	**23**	**+++**	**92**
1.0	1.0	25	19	++++	76
1.0	1.5	25	20	+++	80
1.0	2.0	25	19	+++	76
1.0	2.5	25	13	+++	68

BAP- Benzyl amino purine; Kn- Kinetin; NAA- α Nephthalene Acetic Acid ; (M±SE)= Mean± Standard Error

Response Type-

81< ++++ Excellent ; 61-80 +++ Best ; 41-60 ++ Good
25- 40 + Poor ; >25 - Negligible

A few prominent among them are, *Adhatoda vasica*[16], *Dipteracanthus prostates* (Robert et al. 2012)[17]. Preliminary research on *in vitro* callus production in *B. prionitis* L. has been reported[13,14].

Effect of Cytokinins (BAP and Kinetin: In the Preliminary experiments, the explants responded to BAP better than other cytokines, hence BAP was used. The fungal infection rate of explants varied from 25-40%. A period of surface sterilization exceeding to 5 minutes was found to be lethal to explants. To protect this infection the spray of antifungal agent was done on the explants after inoculation onto MS medium. Earlier, callus induction response was reported in *B. cristata* on basal defined nutrient medium supplemented with Kn and dicamba[15].

After 2 weeks induction response in explants (shoot bud or nodal shoots) was observed on the medium added with BAP alone (1.5mg/L) and Kn (1mg/L), (Table-1; Fig.-1A ,1B). Earlier, a high frequency micropropagation response was observed in *Barleria* using TDZ besides BAP and various auxins. The explants used in this study were Apical, axillary shoots and nodal segments[19].

Effect of BAP, Kinetin combination with NAA: Bud break in explants (shoot tip or nodal segments) were found to be maximum on medium fortified with BAP (1.5) and NAA (1mg/L). Similarly, Kn was also tried in combination with NAA in various

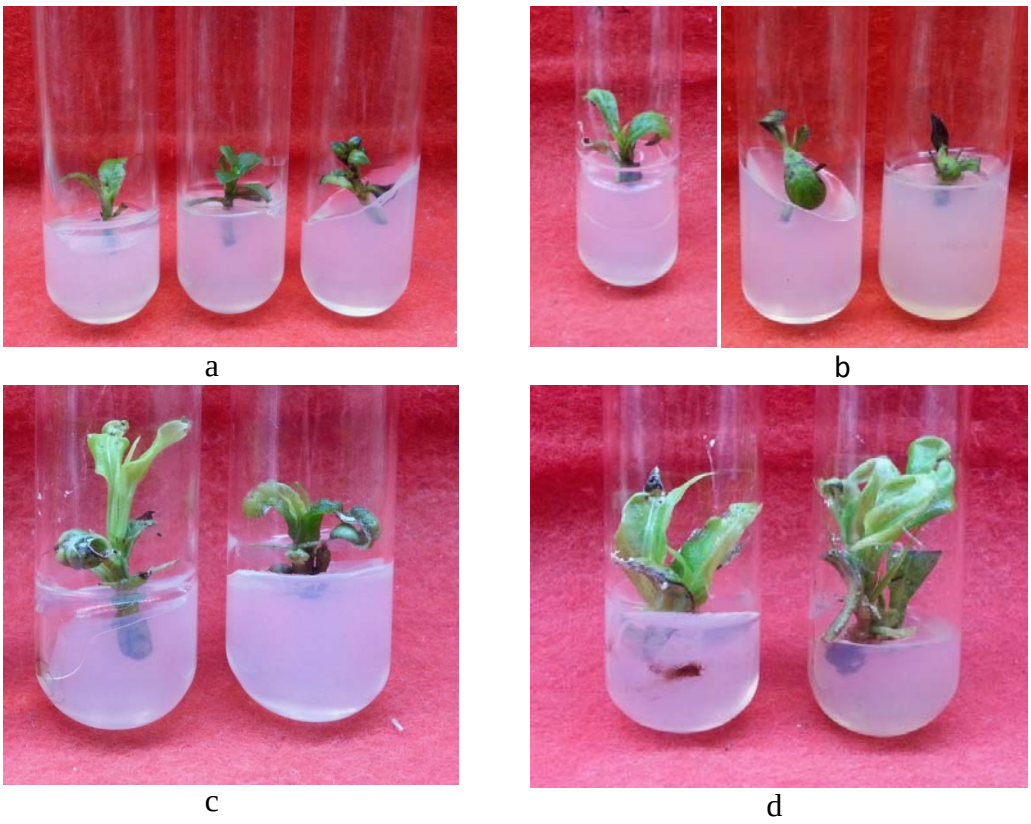

Fig.2. Effect of various hormones on *in vitro* bud breaking from (a) Bud break on MS medium supplemented with BAP (b) Bud break on MS media supplemented with Kinetin (c) Bud break on MS media supplemented with BAP (d) Bud break on MS medium supplemented with Kinetin.

concentrations. It has been found that Kn This best concentration took as further in the combinations of hormones and found maximum response in *Barleria.*

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